

THE ISOLATION AND IDENTIFICATION OF QUERCETIN FROM APPLE PEELS¹

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Some time ago the writer's interest in possible relations between chemical constituents of the apple and storage scald in this fruit led to an investigation of the nonvolatile waxlike substances covering the epidermis.³ This work has been extended to include a study of the yellow flavonol pigment which occurs in apple peels. The present paper deals with the isolation of the pigment and with its identification as quercetin, a widely distributed member of the flavonol group.

The isolation of quercetin has previously been reported from apple bark,⁴ but no mention has hitherto been made of its occurrence in the peel of the fruit.

PREPARATION OF QUERCETIN

The material employed in this work consisted of peels of the McIntosh apple. It was obtained as a by-product in the investigations of Power and Chesnut⁵ on the odorous constituents of apples. In their work, the fresh parings were subjected to distillation in a current of steam. The residue from the steam-distillation was used for the preparation of the flavonol. On account of this treatment and the necessity of prolonged extraction with alcohol, in the presence of malic acid contained in the parings, the glucoside of the flavonol was not obtained.

The wet peels after steam distillation were carefully dried and coarsely ground for purposes of extraction. The extractions were carried out by using a continuous extraction apparatus based on the Soxhlet principle. With this apparatus it was possible to extract about 1 kgm. of material at each loading. The dried material was first extracted separately with petroleum ether and ether in order to remove chlorophyll, carotinoids, and waxlike substances. These extractions were followed by the use of 95 per cent alcohol, which dissolved the coloring matter. The extraction with alcohol was continued for several weeks before the last traces of flavonol pigment were removed from the peels. After evaporation to eliminate the greater portion of alcohol, hot water was added to the syrupy residue. The liquid, upon the addition of neutral lead acetate solution yielded a precipitate which contained very little of the pigment. The lead precipitate was filtered off by means of a Buchner funnel and then basic lead acetate solution added to the filtrate. The basic lead acetate precipitate, which contained most of the coloring matter, was separated by filtration and decomposed by boiling with 5 per cent sulphuric acid. After removal of lead sulphate and cooling, the acid solution was repeatedly shaken with ether in a separatory funnel. Evaporation of the ethereal liquid left a residue consisting of crude

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² In connection with this work thanks are given to Dr. Frederick B. Power for his kindness in supplying the material employed.

³ SANDO, C. E. CONSTITUENTS OF THE WAX-LIKE COATING ON THE SURFACE OF THE APPLE. *Jour. Biol. Chem.* 56: 457-468. 1923.

⁴ ROCHLEDER, F. QUERCITRIN AND QUERCETIN. *Jahresber. Fortschr. Chem.* 1867: 731-732. 1869.

⁵ POWER, F. B., and CHESNUT, V. K. THE ODOROUS CONSTITUENTS OF APPLES. II. EVIDENCE OF THE PRESENCE OF GERANIOL. *Jour. Amer. Chem. Soc.* 44: 2938-2942. 1922.

coloring matter which weighed 2.45 gm. in the air-dried state. This quantity of impure pigment was obtained from 68.6 kgm. of fresh skins, which represented 545.7 kgm. of whole apples. After purification of the crude material only about 0.5 gm. of pure pigment remained.

IDENTIFICATION OF QUERCETIN

Acetylation of the unidentified substance was carried out by boiling the impure pigment with anhydrous sodium acetate and acetic anhydride. The reaction mixture was poured into water and set aside over night. The product thus obtained, after several recrystallizations from 70 per cent alcohol, amounted to 1.15 gm. It consisted of a feltlike mass of colorless needles which melted at 194° to 196° C. When the substance was mixed with pure authentic penta-acetylquercetin its melting point was not altered. The substance was thus identified as penta-acetylquercetin. Combustion of the anhydrous compound dried at 160° confirmed this conclusion.

0.1489 gm. gave 0.3196 gm. CO₂ and 0.0531 gm. H₂O.

Found: C = 58.53, H = 3.99.

Acetylquercetin, C₁₅H₅O₇(C₂H₃O)₅, requires: C = 58.57, H = 3.93.

From the penta-acetylquercetin the free pigment was regenerated by hydrolysis with a few drops of sulphuric acid in glacial acetic acid. After boiling a few minutes, the mixture was well diluted with water and set aside in the ice box for several days. The insoluble compound was collected in a weighed Gooch crucible and dried at 130°.

0.8436 gm. gave 0.4984 gm. recovered pigment.

Found: Pigment = 59.08.

Acetylquercetin, C₁₅H₅O₇(C₂H₃O)₅, requires: C₁₅H₁₀O₇ = 58.98.

The substance obtained by regeneration from its acetyl derivative responded to all the tests of pure quercetin. Crystallized from dilute alcohol, it formed a glistening mass of yellow needles which were almost insoluble in water, but readily soluble in alcohol. Solution in dilute alkalis intensified the yellow color. With alcoholic ferric chloride it gave a dark-green color. When reduced in alcoholic solution with magnesium ribbon and hydrochloric acid it developed a characteristic bright red anthocyanic color. Analysis of the substance, dried at 130°, showed that it agreed in composition with quercetin.

0.1559 gm. gave 0.3399 gm. CO₂ and 0.0466 gm. H₂O

0.1325 gm. gave 0.2891 gm. CO₂ and 0.0379 gm. H₂O

Found: C = 59.46, 59.50; H = 3.35, 3.21

Quercetin, C₁₅H₁₀O₇, requires: C = 59.59, H = 3.34.

DISCUSSION

There is some reason for assuming that quercetin in apple peels may be the chromogenic substance which is responsible for the formation of brown pigment of scalded apples. The investigations of Nagai⁶ have shown that the color of aqueous or alcoholic extracts of numerous plant tissues rich in flavones changes to brown or reddish-brown when the extracts are treated with freshly prepared plant juice containing oxidizing enzymes. This color transformation is attributed to the oxidation of certain flavone and flavonol pigments or their glucosides and in accordance with this view Nagai has shown that chemically pure preparations of the pigments themselves yield brownish colors by the action of enzymes. For example, the addition of oxidase to quercetin resulted in the development of a

⁶ NAGAI, I. A GENETICO-PHYSIOLOGICAL STUDY ON THE FORMATION OF ANTHOCYANIN AND BROWN PIGMENTS IN PLANTS. Jour. Col. Agr. Imp. Univ. Tokyo 8:1-92, illus. 1921.

deep red color which rapidly changed to brown. Quercitrin, a monorhamnoside of quercetin, under the same conditions yielded a less bright red color than quercetin. It was shown that the brown pigment developed at the expense of flavonol or its glucoside in solution. These results with quercetin or its glucoside and oxidase suggest that the brown pigment appearing in scalded apple peels may be produced by interaction in the tissues of quercetin and oxidase which are normally present in the skin. The observation that brown discoloration of scalded peels is more prevalent on the greener portions lends support to the possibility of quercetin or its glucoside being the parent substance of the brown pigment. The bright-red areas are highly resistant to scald and there is reason for believing that flavonol occurs in smaller quantities in these localities because of its conversion into red or anthocyanic pigment.

It should be borne in mind, however, that other substances in the apple may play a part in the formation under certain conditions of brown or reddish brown color. According to Overholser and Cruess⁷ the darkening of cut surfaces of apple tissue may be due to the oxidation of a tannin-like substance. It is interesting to note in this connection that flavonols and tannins are built up of similar nuclei and hence may yield closely related brown products on oxidation.

SUMMARY AND CONCLUSIONS

(1) One of the first indications of scald in apples is the appearance on the greener portions of the fruit of a typical brown discoloration. A possible relation between the suspected occurrence of a flavonol pigment in the skin and scald led to the isolation and identification of this pigment.

(2) The particular flavonol coloring matter, which was found to occur in McIntosh apple peels, proved to be quercetin, $C_{15}H_{10}O_7$. It was identified by means of its penta-acetyl derivative and by combustion of the purified pigment itself.

(3) It is suggested, as a basis for further investigation, that quercetin, or its glucoside which has not been isolated, may be the chromogenic substance which is responsible for the formation of brown pigment in the peels of scalded apples.

⁷ OVERHOLSER, E. L., and CRUESS, W. V. A STUDY OF THE DARKENING OF APPLE TISSUE. *Calif. Agr. Exp. Sta. Tech. Paper* 7: 40 p. 1923.

